Membrane proteins: **Aquaporins – channels without ions**

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Recently determined structures have shed new light on the way that aquaporins act as passive, but selective, pores for the transport of small molecules – such as water or glycerol – across membranes.

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The aquaporins are a large family of integral membrane proteins with interesting properties [1]. The family is divided into the aquaporins proper and the aquaglyceroporins. Aquaporins proper facilitate movement of water across membranes, whereas aquaglyceroporins are specific for glycerol and related compounds. In both cases, the flux of water or other transported species across the membrane is orders of magnitudes faster than for other classes of transporter proteins. It is estimated that water moves through the aquaporin proper AQP1, for example, at a rate of approximately 10^9 water molecules per second per protein molecule.

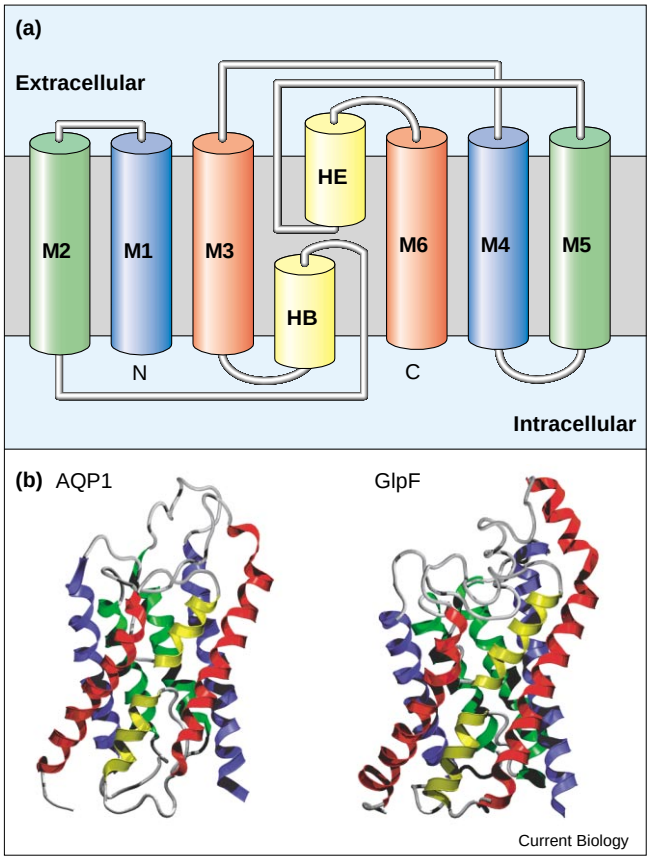
The high fluxes through the aquaporins are indicative of a passive pore or channel across a membrane, rather than of a protein that requires a (slow) conformational change to bring about transport of a solute. Indeed, assuming a pore of radius 2 Å and length 30 Å, and taking into account the expected reduction in diffusion coefficient of water within such a narrow pore [2], one would predict a diffusional flux of approximately 0.5×10^9 water molecules per second [3] — remarkably close to that observed experimentally. This rate should be contrasted with the maximum transport rate of an ion pump of approximately 300 sec^{-1} and of an ion channel of approximately 10^8 sec^{-1}.

Members of the aquaporin family are widespread, occurring in organisms ranging from bacteria to plants and animals. The physiological roles of the ten different mammalian members of the aquaporin proper subfamily have been studied in some detail [4], and a number of disease states have been found to be associated with changes in the properties of the aquaporins proper. Recent studies [5,6] have yielded structures for proteins representing both major branches of the aquaporin family. The high-resolution X-ray crystal structure has been determined [5] of the bacterial glycerol transporter protein GlpF, and medium-resolution electron microscopy (EM) images have been obtained of the mammalian water transporter AQP1 [6,7]. GlpF and AQP1 have similar backbone folds, but differ in their specificity. Detailed comparison of the two structures has provided important clues as to the molecular origin of this difference in specificity.

Protein structures

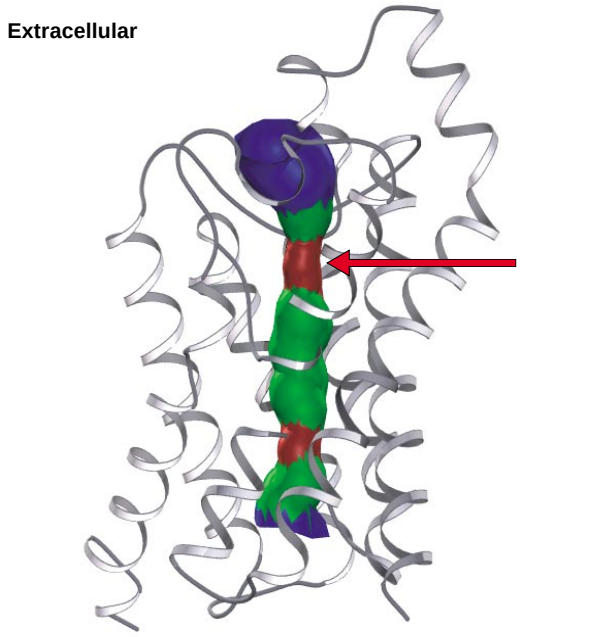
The transbilayer topology of members of the aquaporin proper family (Figure 1a) has been established in advance of structure determination by indirect methods, such as transmembrane helix prediction and mutational analysis [8]. The new structures [5–7] show that the predicted topology is correct. Both proteins have six transmembrane helices and two 'half-transmembrane' helices that 'dip

Figure 1



(a) Transmembrane topology of an aquaporin. Note how the helix–helix–loop–helix motif (coloured blue–green–yellow–red) is repeated twice (M1–M2–HB–M3 and M4–M5–HE–M6), giving an element of twofold pseudo-symmetry within the fold. The membrane is represented by a grey band. (b) A comparison of the folds of AQP1 [7] and GlpF [5], using the same colour scheme for helices as in (a).

Figure 2



The geometry of the GlpF pore [5]. The backbone of GlpF is shown as a grey ribbon. The lining of the pore – determined using HOLE [13] – is shown as a solid surface, for which red indicates the pore radius is less than that of a water molecule, green indicates the pore radius is equivalent to that of one or two water molecules, and blue indicates the radius is greater than that of two water molecules. The red arrow indicates the location of the constriction caused by the sidechain of tryptophan 48.

into' the membrane. In some respects, the latter motifs are reminiscent of the P-loops of potassium channel subunits [9]. In both cases, the transmembrane pore is lined by re-entrant loops that help to confer specificity. In the aquaporins proper, however, one subunit provides two loops to the pore — consistent with its two-fold internal pseudo-symmetry (see below) — whereas in the case of potassium channels, four subunits each contribute one P-loop to the pore.

As mentioned above there is a degree of internal symmetry within the topology of aquaporins proper (see Figure 1a), suggesting that their early evolution involved gene duplication. The two re-entrant loops are between helices M2 and M3 (loop HB), and between M5 and M6 (loop HE). The first loop, HB, is from the intracellular surface of the protein, and the second loop, HE, from the extracellular surface. Both loops contain a characteristic asparagine–proline–alanine (NPA) sequence motif that is highly conserved within the aquaporin family. In the water-transporting aquaporins there is a cysteine in the extracellular HE loop which is responsible for the inhibition of water transport by $HgCl_2$. There is also a histidine residue that is conserved within the aquaporins proper, but is replaced by a glycine in the aquaglyceroporins. This residue is therefore proposed to play a role in specificity of aquaporins for water.

The aquaporin fold is quite complex, with the six transmembrane helices forming a 'permuted ring', within which are embedded the two loops that form part of the lining of the central pore. The helices are highly tilted relative to the lipid bilayer normal — at an angle of approximately 25–35° — and form a right-handed supercoil. Glycine residues play an important role in helix–helix contacts, as has been suggested to be the case for a number of membrane proteins [10]. As in many membrane proteins, the location of the protein with respect to the lipid bilayer appears to be determined by tryptophan and tyrosine residues on the protein surface, close to the presumed location of the lipid headgroups. There do not appear to be any significant differences between the folds of AQP1 [6,7] and GlpF [5].

From structure to function

The newly determined aquaporin structures [5,7] make it possible to propose a preliminary mechanism for aquaporin function that explains, at atomic resolution, the physical basis of transport and selectivity. In this context, it is of particular importance that the GlpF structure [5] was determined from crystals grown in the presence of approximately 2M glycerol. The GlpF molecule in the crystal has three glycerol molecules — plus an intervening water molecule — within its pore.

The first clue to the structural basis of aquaporin function comes from examining the shape and size of the transbilayer pore (Figure 2). The pore dimensions in GlpF [5] and AQP1 [7] are broadly similar, apart from a somewhat wider central cavity in the GlpF pore than in the AQP1 pore. In GlpF, the pore is approximately 30 Å long and has a mean radius of about 2 Å. It is constricted towards the extracellular mouth of the pore by the sidechain of residue tryptophan 48, which lies on the other side of the central pore from the NPA motif of loop HE, in a region that Fu *et al.* [5] have designated the 'selectivity filter'. It is suggested that this is the region that allows GlpF to discriminate between glycerol and its analogues, to which GlpF is highly permeable, and water, which does not pass readily through the GlpF pore.

In AQP1, the equivalent residue to tryptophan 48 in GlpF is isoleucine 60, which forms part of a ring of hydrophobic residues that appear to constrict the pore in this region [7]. The region close to the centre of the AQP1 pore, where the asparagine side chains of the two NPA motifs project into the pore, has been suggested to be responsible for

preventing H_3O^+ from permeating. The asparagine side chains are proposed to do this by forming sidechain–water hydrogen bonds which disrupt the formation of a continuous hydrogen-bonded water wire, such as would be needed for rapid conduction of protons via the Grotthüs mechanism [11].

Given their mean radius of about 2 Å, why do ions not pass through the GlpF or AQP1 pores? The long, narrow pore of these proteins could not accommodate a *hydrated* ion. In the case of potassium channels, the selectivity filter region is narrow but it can accommodate a *dehydrated* K^+ ion by replacing water molecules with peptide backbone oxygen atoms that solvate the ion. The GlpF and AQP1 pores, in contrast, do not provide residues that could fully solvate permeant ions. In both GlpF [5] and AQP1 [7], the pore lining is amphipathic, with approximately one half of the pore-lining surface made up of hydrophilic groups and the other half made up of hydrophobic groups. In GlpF [5], the glycerol molecules are oriented so that their hydroxyl groups form hydrogen bonds to the polar half of the pore lining, whilst their carbon atoms interact with the apolar half of the lining. One can envisage how such a pore favours glycerol permeation.

A similar pore lining is found in AQP1 [7], where the bulk of the pore is amphipathic, apart from a predominantly hydrophobic region around the constriction site. In the latter case, it is not immediately clear why this should support the observed high rate of water permeation. From the pore dimensions it is clear that only a single file of water molecules can be accommodated in AQP1, whereas in GlpF potentially more waters could be accomodated in the central 'cavity'. But it is not entirely clear how this difference is related to the difference in selectivity of these two closely related pore molecules.

The future…

It is very encouraging that a number of structures of membrane transport proteins from different families — channels, pumps, aquaporins — are starting to emerge. At last, there is a real possibility of understanding membrane transport processes at atomic resolution. Of course, whilst a high-resolution structure provides valuable clues as to the mechanism of a transporter, it is not the last word. Rather, access to an atomic or near atomic resolution structure enables one to formulate more detailed mechanistic questions than was previously possible. In particular, we would like to understand in more detail *why* the rates of water and glycerol permeation through their respective aquaporins are so high, and what is the exact basis of, for example, the selectivity for glycerol or water. Taking potassium channels as a paradigm [12], it would seem that computer simulations of water/glycerol movement through AQP1/GlpF pores are likely to play an important part in such investigations.

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